Biodegradable Inflatable Balloon for Reducing Radiation Adverse Effects in Prostate Cancer

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Abstract: Carcinoma of the prostate is one of the most abundant killers for men in the western world, and it is frequently treated via Radiation therapy. Unfortunately, radiotherapy side effects include rectal irritation and bleeding, erectile dysfunction and urinary frequency. Because radiation intensity decays rapidly as a function of distance, displacing irradiated prostate away from normal tissues would reduce damage and therefore side effects. The objective of this study is to develop an inflatable balloon that is implanted via a minimal invasive procedure. The balloon is made of a biodegradable polymer called poly(lactide-co-e-caprolactone). The implant is inserted rolled throughout the perineum; inflated in situ with a physiological saline; sealed and placed between the rectum wall, and the prostate gland. Balloon’s mechanical and chemical properties were extensively characterized both in vitro and in vivo. The balloon’s preparation ensures no bonding across surfaces as these may endanger the implant mechanical stability. Moreover, the coating method does not alter the polymer's molecular weight and therefore preserve its mechanical properties. Balloon’s sterilization was carried out using ethylene oxide which, as our results show and in comparison with α-irradiation, doesn’t damage the mechanical stability of the implant. The proper functionality of the insertion-mounting device as well as the balloon capability to retain its inflated form during patients’ radiation session was demonstrated both in vitro and in vivo. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 91B: 855–867, 2009

Keywords: prostate cancer; balloon barrier; radiation protection; radiotherapy; biodegradable implant

INTRODUCTION

Carcinoma of the prostate is one of the most common tumors affecting men in the western world. Approximately 220,000 new cases are diagnosed each year in the United States alone.1 Several therapeutic options are available for treating prostate cancer, including watchful waiting, radical prostatectomy, radiation therapy, cryosurgical ablation of the prostate, hormone therapy, and systemic chemother¬apy.2 Radiation therapy is delivered primarily using an external-beam technology or brachytherapy methodologies.2 Unfortunately, the adverse effects of radiotherapy significantly reduce the patient’s quality of life. Side effects include erectile dysfunction, rectal irritation and bleeding, and increased urinary frequency.3 The adverse effects result from the prostate’s proximity to the rectal wall and the nearby innervation areas. It is hypothesized that because radiation intensity decays as a function of distance, by temporarily displacing the irradiated prostate away from the most sensitive normal tissues, mainly the rectum wall and nearby innervation areas, it is possible to minimize clinical dysfunctionalities associated with irradiation. Polymers are frequently used in the preparation of biomedical implants. Polymer application include medical sutures,4,5 antiwear coating of orthopedic implants,6,7 bioactive coating,8,9 drug-controlled release carriers,7,10 dental implants,11,12 anti-adhesion films,13,14 and the preparation of scaffolds for tissue engineering.15,16 Durable polymers have a stable chemical structure and their overall physicochemical properties remain intact under physiological conditions.17 Because their stagnation properties are not suitable for applications that require only a temporary functionality,18 chemists have developed numerous polymers that degrade and absorb in the body. These materials are referred to as
biodegradable polymers. Typically, the molecular weight (MW) of a biodegradable polymer and its mechanical stability decrease as a function of time. Biodegradable polymers continue to degrade until polymer chains are small enough to engage in the body metabolism. Poly(lactic acid) (PLA), a biodegradable polyester, is an FDA-approved polymer. The molecular structure of PLA, and of similar poly(γ-hydroxy acids), contains unstable ester groups and therefore their degradation is a simple hydrolytic process. When a PLA chain reaches a MW of about 2000 Da, it is engaged in the “Krebs cycle” and degrades into H₂O and CO₂. The mechanical properties of a polymer depend on many factors, such as its chemical structure, MW, and unique preparation methodology. The situation of a biodegradable polymer such as PLA is more complicated because its mechanical properties change gradually over time. Typically, during the initial degradation phase, the PLA shows no significant mass loss and the mechanical properties of the implant decline in a gradual manner. However, the MW of the polymer decreases to several thousands Daltons, it undergoes extensive weight loss. Usually, a decrease of merely 5% in the implant mass leaves it with no mechanical properties.

Several methods are available for sterilizing medical implants, including dry heating, autoclaving, γ-irradiation, and exposure to gaseous chemicals. When selecting an adequate sterilization process for a medical implant, one must take into account its clinical functionality. Most of the sterilization methods listed earlier are used under extreme conditions. Therefore, sterilizing polymers can result in an undesired reduction of their physical and mechanical properties. γ-Irradiation is a simple and short process both in practice and in the required regulatory pathway, but it results in a reduction of up to 50% of the polymer’s MW and a significant deterioration of its mechanical properties. Sterilization processes that utilize exposure to such chemicals as ethylene oxide (EtO) require the diffusion of the gas throughout the implant. It is a highly efficient process, but it requires thorough validation. This procedure must ensure that no poisonous gas residues remain entrapped within the polymer. Although EtO exposure requires a longer regulatory pathway, the method usually does not alter the mechanical properties of the polymer, unless a particular chemical reaction takes place between the two.

Several balloon-shaped implants have been described recently in the literature. Balloons were utilized as hydrophilic osmotic hydrogel expanders, intragastric balloons for patients suffering from obesity, minimally invasive antral membrane balloons, and microballoons for intrinsic sphincter deficiency. These unique platforms use their volume to answer a specific clinical necessity. Balloon implants are usually prepared from durable polymers with permanent mechanical properties. The objective of this study was to examine the functioning of an inflatable balloon system inserted through a minimally invasive proce-
DCM. Subsequently, a second layer was dip coated on top of the first one. Three cycles of coatings were used to achieve a desired balloon wall thickness of 100 μm. Balloons made with lower wall thickness of 50 and 75 μm were made using polymer solution having a concentration of 9 and 12% w/v, respectively. Agarose was removed by immersing the coated cast in double distilled water (DDW), preheated to 90°C for a period of 2 min until it became fluidic and could be gently extracted out of its nozzle. Nine washing cycles were carried out by injecting 5 mL of hot DDW through the balloon’s nozzle. The balloon was then dried and evaluated for its weight and average wall thickness.

**Sleeve and Plug Preparation.** Biodegradable balloon nozzle was straightened with a PLCL made sleeve. Sleeves were designed to have an outer diameter of 2.5 mm and an inner diameter which varied between 2.0 and 1.5 mm. Stepped inner diameters enabled a designated plug to be stacked in the sleeve, thus sealing the balloon. The sleeve was prepared using a PLCL solution by a dip-coating technique. Metallic templates were immersed into the polymer solution having a concentration of 20% w/v PLCL/DCM. To achieve a desired outer diameter, four coating cycles were carried out. Sleeves were joined onto the balloons nozzle using a thin gluing film of the 20% w/v polymer solution. PLCL plug was fabricated using an appropriate metallic mold. The polymer was heated up to its melting point and then compressed into the mold. The final balloon system was put into a vacuumed desiccator for at least 7 days to evaporate DCM residues.

**Balloons’ Designated Catheter System.** An installation kit that allows insertion, inflation, and seal by a minimally invasive procedure was developed (see Figures 1 and 3). The kit includes a needle, guide wire, a 3–4 mm dilator with a sheath passed over the dilator, and the folded balloon inside a second sheath that can be introduced through the dilator sheath. The needle, guide wire, and dilator are commonly used as part of a method known as the Seldinger technique. This minimally invasive technique is used to create open access for a device or material through a sheath to a certain location in the body. When the sheath is put in place, the balloon is deposited, inflated, and sealed. (Folded inside packaging sheath, see Figure 3). At that point, the balloon is exposed by retracting both sheaths and inflated at the proper location and orientation. The balloon is then deployed by inserting physiological solution to the desired volume and then sealed to prevent deflation by using a biodegradable plug that will be forcefully stuck into a nonelastic biodegradable sleeve situated at the orifice of the balloon. The inflated balloon is expected to maintain its shape and location for the duration of the radiation therapy up to 6 weeks.

**Agarose Residues Quantification.** Agarose residual quantity in PLCL balloons was determined to validate its washing procedure. Following agarose removal procedure, nonsterilized PLCL balloons were rinsed with 5-mL aliquots of 90°C DDW. Each balloon was rinsed nine times and the medium was collected for further analysis. Balloons were then cut into four pieces and rinsed in 90°C DDW (40 mL) for a period of 120 min, the medium was collected. Rinsing volumes were quantified for the amount of residual agarose. Measurements were performed by the phenol-sulfuric acid method. Calorimetric spectra were taken on Konton Instrument Uvicon model 930 (Msscientific, Berlin, Germany); absorbance was read at 490 nm. Measurements were performed in duplicates and compared with a calibration curve obtained at similar conditions.

**Dichloromethane Residues Quantification.** Two samples of bulk PLCL and two nonsterilized PLCL balloons were analyzed for their DCM residual quantities. The analysis was performed at a third party authorized laboratory (TAMI Institute for research and Development Ltd., Haifa, Israel).
Israel). The analysis was based on gas chromatography/mass spectroscopy (GC/MS) with headspace injection technique. Assays were prepared by 50-min extraction of the samples in a concentrated NaCl solution heated to 90°C.

**Sterilization.** PLCL balloons sterilization process was carried out using two well-known sterilization methodologies: γ-irradiation and EtO exposure. γ-Irradiation was performed at Sorvan radiation Ltd., Israel. Irradiation was conducted with a dose of 2.5 Mrad. EtO exposure was carried out at MediPlast Ltd., Israel. EtO exposure was conducted for 360 min, at 45°C. Both sterilization facilities works under good laboratory practice (GLP) standards for medical devices.

**Balloon Durability**

**PLCL Balloon’s Intactness Profiles.** Intactness evaluation is a measure that was developed to assess the physical stability PLCL balloons incubated in physiological conditions. All samples were inflated with isotonic saline, and sealed using the novel catheter system (see Figure 4). Samples were statically incubated in glass jars filled with a phosphate-buffered saline (PBS), pH 7.4 at 37°C. The PBS medium was refreshed weekly. Intactness evaluation was conducted as following: each week, balloons were removed, wept up, and weighted. Intactness was monitored by gently pressing their dry surface against a wiping paper to detect any possible leakage. A balloon that leaked was removed out of incubation and excluded out from the group. Both group A and B were composed of 10 nonsterilized PLCL balloons having an average wall thickness of either 75 or 100 μm, respectively. Group C was composed of six PLCL balloons having a wall thickness of 100 μm. Balloons of group C were sterilized using the γ-irradiation method. Results are presented as the % of survived balloons as a function of incubation time.

**PLCL Films Weight Loss Profiles.** PLCL films weight loss following incubation at aqueous environment was determined. Nonsterilized PLCL balloons having wall thicknesses of 50, 75, and 100 μm and γ-irradiated balloon having a wall thickness of 75 μm were prepared according to above protocol. Balloons were cut to 40 mg films and then were statically incubated in glass scintillation tubes, filled with PBS, pH 7.4 at 37°C. The PBS medium was refreshed weekly. At each time point, films were removed from the incubation medium and were allowed to dry at ambient conditions for a period of 24 h. Films were weighed using an analytical scale and their weight loss was calculated relative to their initial value.

**PLCL Films Molecular Weight Profiles.** PLCL MW loss because of its hydrolytic degradation in aqueous environment was evaluated for PLCL balloons. Similar samples that were used for PLCL films weight loss profiles served in balloon’s films MW loss evaluation. PLCL films (40 mg) were statically incubated in glass scintillation tubes, filled with PBS, pH 7.4 at 37°C. The PBS medium was refreshed weekly. At each time point, films were removed from the incubation medium and were allowed to dry at ambient conditions for a period of 24 h. MW of the polymers were estimated on a gel permeation chromatography (GPC) system consisting of a Waters 1515 Isocratic HPLC Pump, with 2410 Refractive Index detector(RI) (Waters, MA), a Rheodyne (Coatati CA) injection valve with a 20-μL loop. Duplicate samples were eluted with chloroform through a linear Styragel® HR-4 column, (Waters, MA) at a flow rate of 1 mL/min. The MWs were determined relative to polystyrene standards (Polysciences, Warrington, PA) with a MW range of 12,500–270,000, using BREEZE 3.20 version, copyright 2000 Waters Cooperation computer program.

**PLCL Films Thermal Characterization.** Similar samples that were used for PLCL films weight loss profiles served in balloon’s films thermal evaluation. Thermal analysis was determined on a Mettler TA 4000-DSC differential scanning calorimeter (Mettler-Toledo, Schwerzenbach, Schweiz), calibrated with Zn and In standards, at a heating rate of 10°C/min) under nitrogen atmosphere.

**PLCL Films Mechanical Characterization.** Mechanical properties of PLCL balloons are deteriorating because of their hydrolytic degradation following incubation at physiological conditions; testing of the above was conducted using PLCL Films. PLCL films for mechanical characterization were prepared in a similar manner to balloon’s dip-coating method as was described above. However, instead of balloon shaped molds, polymer films were generated on a flat-shaped agarose molds. Coating parameters were adjusted to prepare films of 100-μm wall thickness, which were then cut to rectangular shaped films: 4-cm length and 1-cm width. Each time point consisted of seven replicates, tested simultaneously for statistics. Samples were statically incubated in glass scintillation tubes filled with PBS, pH 7.4 at 37°C. The PBS medium was refreshed weekly. Nonsterilized samples were tested in the following intervals: 0, 7, 30, 60, and 120 days. In addition, γ-irradiated samples were tested at 0 and 30 days. The tensile properties at room temperature were determined using an Instron® Universal tensile tester model. Films were drawn at a constant velocity of 30 mm/min. Results were statistically evaluated by Turkey-Kramer multiply compression test using GraphPad InStat® version 3.01.

**In Vivo Feasibility Studies**

**Dog Experiment.** One dog 12 years old, 35 kg was implanted with a biodegradable balloon, made of PLCL polymer; prior to procedure, the dog was prepared with a purgative regimen to evacuate the bowels and with prophylactic antibiotics. The dog was positioned supine (on his back) with his hind legs extended toward the head to expose the perineum. The perineum anus and lower rectum were properly cleaned and scrubbed with an antisectic solution. Trans rectal ultrasonography (TRUS) was used in addition to palpation to guide the implantation of the balloon. A transverse incision of 3 mm was performed between the an-
terior aspect of the anus and the perineum. Under TRUS guidance, the perineal membrane was punctured and a space between the rectum and anterior pelvic wall and the posterior aspect of the prostate was inflated by saline injection. Then, a guide wire was introduced through the needle into the prepared space and the needle was pulled out. A dilator was passed over the guide wire to enlarge the tract. The dilator and guide wire were removed leaving the sheath covering the dilator. The deploying device with the folded balloon was introduced through this sheath to the proper position. The sheaths covering the balloons were retracted revealing the balloon. Then the balloon was inflated with 7 mL of sterile saline mixed with iodinated contrast material. At the end of the inflation, the deployment device was disengaged and the balloon was sealed by the plug. The deployment device was removed, and the position and proper inflation of the balloon were ascertained by palpation and by TRUS monitoring (measuring the longitudinal, transverse dimensions of the inflated balloon). The integrity of the rectal wall was ascertained by trans rectal palpation. The incision in the perineum was sutured by one stitch. The experiment goal was to evaluate the proper functionality of the catheter system, including balloon's proper sealing mechanism. Following the procedure, the balloon location and inflated form was evaluated using X-ray imaging. The dog was followed up for 1 year, and during this period, the dog was evaluated periodically and examined by the animal research institute's veterinarian. The dog was anesthetized and sacrificed according to standard procedure. The dog was positioned on its back and the abdomen and thorax were opened. Samples of the lung, heart, liver, spleen, mesenteric lymph nodes, and kidney were excised and were sent for histopathological examination. Samples of the prostate were also sent to histopathological examination.

Guinea Pig Experiment. Four guinea pigs were implanted with a biodegradable balloon, made of PLCL polymer. Balloons were inserted subcutaneously using a minimal invasive catheter system, along with a standard seldinger kit. The balloon was inflated in situ using saline and properly sealed. The experiment goal was to evaluate the proper system functionality and to evaluate in vivo durability of implanted balloons following implantation. The procedure was conducted under anesthesia; animals were than checked daily to validate weight gain and vitality. Following 14 days, animals were sacrificed and balloons were removed for evaluation. Local ethical committee (The Hebrew University) approved both animals' experiments.

RESULTS

Balloon and Catheter System Preparation

A unique methodology was used to prepare balloons because of the requirement that they contain no bonding parts along their surface. Such areas are known to be highly delicate and therefore susceptible to failure. The process combines two fundamental steps: a permutation of "lost wax" casting and a dip-coating technique.

Dip coating was used to "build" the balloon walls by dipping a preshaped model in a solution containing the biodegradable polymer (PLCL), which was dissolved in an organic solvent (DCM). The preshaped model was made of a material that is later extracted from the balloon through its orifice. Agarose, a hydrophilic polysaccharide, served as the casting agent. It was chosen because its hydrogel allows the formation of a PLCL film and because the gel is liquefied easily at relatively low temperatures (between 50 and 90°C). Preparation of the balloon requires successive dip-coating cycles to reach a homogeneous thickness profile.

Figure 2 illustrates the normalized thickness profile of a representative balloon with a 100-μm wall. It was dip coated using a PLCL solution (14% w/v PLCL/DCM) at a rate of 20 cm/min. Two dips were carried out while the template nozzle was turned up, and an additional one was applied while it was turned down. Changing coating direction is needed because the polymeric film flows before the DCM is fully evaporated.

Coating was carried out in a working chamber operated with a constant nitrogen flow. Drying of the chamber was a necessary step in the multi-cycles dip-coating process. As the DCM evaporates from the applied polymeric film, a substantial decrease in its surface temperature occurs, and the nitrogen flow ensures that water does not condense onto the generated PLCL film. In an uncontrolled atmospheric environment, the process would result in the condensation of water droplets. The condensate generates small bubbles of 0.5–1 mm in the balloon surface. Bubbles are vulnerable points because of the reduced thickness in the region of the bubble relative to the average wall thickness. Therefore, balloons prepared in a humid environment are usually dripping when inflated with physiological saline.

![Figure 2. Normalized thickness profile of PLCL balloon prepared via a dip-coating technique. Balloons with an average diameter of about 100 μm were prepared at dipping velocity of 20 cm/min, using 14% w/v PLCL/DCM solution. Coating was carried out using three dipping cycles, two of which while the nozzle is headed upward.](image-url)
Residues of agarose and DCM, two of the main substances used in the preparation process, were quantified. Agarose quantification was conducted using a well-known phenol-sulphuric acid method. Agarose was dissolved at 90°C DDW and then removed through the balloon orifice; its residual quantities were repeatedly washed with hot water. Washed volumes were collected and analyzed.

Table I shows the residual quantities of agarose in manufactured PLCL balloons. As agarose was gradually removed, after five washing steps extract quantities decreased below the detection limit of the method. In addition, to evaluate the implant itself, clear and washed balloons (after nine washing steps) were cut into several pieces and incubated in hot water for a period of 1 h. Testing the extract determined that less than 0.05% w/w of agarose was left in the final balloon implant (<500 ppm).

Residual quantities of DCM in manufactured balloons and in samples of bulk polymer material were measured in duplicates using a GC/MS apparatus. DCM values in manufactured balloons had a residual value below the detectable limits of the assay (<0.3 ppm).

### Table I. Residual Quantities of Agarose in Fabricated PLCL Balloons

<table>
<thead>
<tr>
<th>Agarose Quantification (Wash Number)</th>
<th>Agarose Average Weight (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (initial extraction)</td>
<td>355,700 (99,840)</td>
</tr>
<tr>
<td>2</td>
<td>10,340 (6,500)</td>
</tr>
<tr>
<td>3</td>
<td>2550 (3070)</td>
</tr>
<tr>
<td>4</td>
<td>110 (50)</td>
</tr>
<tr>
<td>5–10</td>
<td>Out of range</td>
</tr>
<tr>
<td>Residue</td>
<td>160 (30)</td>
</tr>
</tbody>
</table>

Balloon was mounted on the designed catheter system, as shown in Figure 3. The balloon was glued onto a PLCL sleeve that was already mounted on the catheter needle. A PLCL sealing plug was also attached to the edge of the catheter’s middle. Plug diameter and shape were adjusted to fit perfectly the interior architecture of the sleeve to ensure its sealing properties.

Figure 4 illustrates the balloon inflation process. The balloon was rolled up on the catheter (A), inflated with saline (B, C), and then by pushing the plug into the sleeve cavity it was sealed (D, E).

**In Vitro Chemical and Physical Evaluation of the Balloon**

The most critical requirement for the balloon is to preserve an inflated shape, because it is intended to distance the prostate from healthy tissues throughout the patient’s radiation program. To test this property, an in vitro evaluation of the balloon’s functionality was developed (intactness profile). Balloons were incubated under physiological conditions, in 0.1M PBS pH 7.4. Implant stability was monitored for a period of 120 days. Each week, balloons were removed from the medium, wiped, weighted, and tested for leakage. Three experimental groups were used to characterize intactness profiles: Group A comprised nonsterilized balloons with wall thicknesses of 75 μm. Group B comprised nonsterilized balloons with wall thicknesses of 100 μm. Group C balloons were subjected to γ irradiation and had wall thicknesses of 100 μm.

Figure 5 shows the intactness profiles of nonsterilized and γ-irradiated balloons. Survival of a balloon means that it was fully inflated with no detectable leakage. The integrity of nonsterilized balloons was correlated to their wall thickness. Nearly 50% of the 75-μm group balloons failed after 1 week, but the 100-μm group failed only after 90 days of incubation. Moreover, the first leaking balloon in group B was detected after 7 weeks. γ-Irradiated balloons showed lower survival than the nonsterilized samples, and the entire group C failed in less than 3 weeks (not meeting basic implant requirements). The complete data regarding group B, nonsterilized, 100-μm balloons is shown in Table II.
Weight loss records of the inflated balloons support the intactness profiles of the balloons, as no significant weight loss was found during incubation. It is important to note that after 7 weeks, the weight loss data corresponds only to the “survived” samples.

Weight loss evaluation was conducted using balloons that were cut into 40 mg pieces. Testing compared four different groups: groups A, B, and C comprised nonsterilized balloons with wall thickness of 50, 75, and 100 µm, respectively; group D samples comprised γ-irradiated balloons with a wall thickness of 100 µm. All samples were incubated under physiological conditions and their weight and physical stability was recorded for a period of up to 160 days.

Figure 6 summarizes the weight loss profiles of nonsterilized and γ-irradiated PLCL balloon films. In the first 8 weeks, no significant differences were observed between the nonsterilized groups. During the same time, nonsterilized films lost 0.35, 0.65, and 0.75% of their weight in the 50, 75, and 100-µm groups. At 138 days of incubation, 100-µm films lost only 2.3% and 50-µm films lost over 4% of their initial weight. Nonsterilized films become brittle after about 140 days; the sudden increase in standard deviation values is an indication of films disintegration. By contrast, γ-irradiated samples incubated under similar conditions presented a much steeper degradation profile than did nonsterilized balloons. After 8 weeks at physiological conditions, weight loss of γ-irradiated films was four times greater than that of nonsterilized samples. γ-Irradiated films also began to disintegrate after 72 days, compared with 150 days for the nonsterilized ones.

Table III shows the PLCL MW loss for nonsterilized 50 and 100-µm balloons. MW decreased linearly regardless of wall thickness. An increase in polymer polydispersity index (PDI) was recorded until 117 days for both thicknesses. By contrast, at the last measurement point PDI suddenly decreased. A decrease in PDI can indicate extensive release of low MW polymeric chains into the incubation medium. Table VI describes the MW loss of γ-irradiated films compared with nonsterilized ones. In addition to the significant decrease in initial MW from 93 to 63 kDa, degradation rate was significantly higher for the sterilized samples. The PDI index of the γ-irradiated samples began to decrease at 72 days, a change in trend that occurred at a much earlier stage than in nonsterilized samples, indicating again the significant weight loss of low MW fragments.

In addition to the intactness profiles and other analyses described above, extensive studies were performed on the mechanical characterization of PLCL films as a function of time. The balloons’ mechanical properties were tested using PLCL films prepared with the dip-coating method. Films were prepared using an agarose template that was dip coated in PLCL solution, followed by a template removal procedure conducted in hot water. To generate homogeneous and

### Table II. In Vitro Weight Loss Profiles of PLCL Balloons

<table>
<thead>
<tr>
<th>Incubation Time (Days)</th>
<th>Normalized Average Inflated Balloon’s Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.68 (0.14)</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>99.99 (0.19)</td>
</tr>
<tr>
<td>21</td>
<td>100.01 (0.21)</td>
</tr>
<tr>
<td>35</td>
<td>99.97 (0.23)</td>
</tr>
<tr>
<td>49</td>
<td>99.90 (0.27)</td>
</tr>
<tr>
<td>62</td>
<td>99.69 (0.34)</td>
</tr>
<tr>
<td>76</td>
<td>99.66 (0.29)</td>
</tr>
<tr>
<td>90</td>
<td>99.60 (0.32)</td>
</tr>
<tr>
<td>104</td>
<td>99.35 (0.07)</td>
</tr>
</tbody>
</table>

Figure 5. Intactness profiles of PLCL balloons. Balloons having a wall thickness of 75 and 100 µm were inflated with saline, and then sealed using its catheter system. Nonsterilized and γ-irradiated balloons were statically incubated in isotonic PBS, pH 7.4, 37 °C. Samples were weekly monitored for their total weight and for any possible leakage.

Figure 6. PLCL films weight loss profiles. Nonsterilized and γ-irradiated balloons films having a wall thickness of 50, 75, and 100 µm were statically incubated in isotonic PBS, pH 7.4, 37 °C. Weight loss of the films was monitored on a weekly basis.
relatively long films, templates made of rectangular agarose surfaces were selected rather than balloon shaped ones. Films were cut to pieces of 1 cm × 4 cm × 100 μm (width × length × thickness). Samples were incubated at physiological conditions for various time periods.

Figure 7 shows the mechanical properties (stress test) of nonsterilized and γ-irradiated films following 0, 30, 60, and 120 days of incubation at physiological conditions. 100-μm PLCL films tested immediately after preparation (0 days) were highly flexible and capable of elongation of nearly 650% of their original length (testing was conducted at a velocity of 30 cm/min). All measurements were normalized relative to day 0 values obtained for nonsterilized PLCL films. Nonsterilized balloon films preserved the majority of their mechanical properties such as energy to break, ultimate stress, and elongation during the first 60 days of incubation (55.6, 63.5, and 63.6%, respectively). After 120 days, the mechanical properties of nonsterilized films were less than 10% of their initial values. The mechanical properties of the γ-irradiated films showed a substantial loss of their properties compared with nonsterilized samples. Figure 7 shows a decrease of 40.7, 35.8, and 26.4% in the films’ energy to break, ultimate stress, and elongation. These measurements were conducted immediately after sterilizing the samples (day 0) and were found to be statistically relevant. A significant loss in the mechanical properties of γ-irradiated films was recorded early, after only 30 days of incubation, when films lost 96.3, 58.3, and 95.3% of their energy to break, ultimate stress, and elongation. These mechanical measurements were found to be statistically significant relative to their nonsterilized counterparts, which had decreased by 22.2, 9.0, and 27.1% after 30 days of incubation. The above changes in balloon properties are shown in Figure 8(A): 30 days after γ-irradiated balloons were incubated, several cracks appeared spontaneously along stress lines. These cracks, nearly 0.5 cm long, indicate that the γ-irradiated polymer had lost its flexibility entirely.

Polymeric substances can be subject to changes in their chemical and physical properties as a function of time during incubation. In the case of biodegradable polymers, such as PLCL, biodegradation definitely causes such a change. Investigating the polymer’s thermal properties is therefore important and can be related to other chemical and physical data. Nonsterilized balloons with a wall thickness of 50, 75, and 100 μm were cut into 40 mg pieces and incubated under physiological conditions for a period of 104 days. Table IV summarizes a differential scanning calorimetry analysis conducted for nonsterilized balloon films. Seventy-five micrometers films showed a change in the polymer’s thermal properties as a function of time. The material’s heat capacity increased in absolute value from 223.51 J/g (0 day sample) to 27.03 J/g (104 days sample). This change was gradual and was observed all along the incubation. In addition, films changed their transparency and became rather more opaque. The changes in thermal properties and in color may be an indirect indication of an increase in the polymer crystallinity. Similar results for both heat capacity and transparency were obtained for the 50 and 100-μm PLCL balloon films.

Studies of balloon durability, including their intactness profile (Figure 5), weight loss (Figure 6), MW loss (Table III), and mechanical properties (Figure 7) showed the deteriorating effect of γ-irradiation on the implant properties, which shortens significantly its potential period of functionality. Consequently, additional tests were conducted to

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>M_w (Da)</th>
<th>M_n (Da)</th>
<th>PDI</th>
<th>M_w (Da)</th>
<th>M_n (Da)</th>
<th>PDI</th>
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<tr>
<td>0</td>
<td>114,845 (368)</td>
<td>86,554 (1,118)</td>
<td>1.33 (0.01)</td>
<td>115,167 (1389)</td>
<td>87,956 (473)</td>
<td>1.31 (0.02)</td>
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<td>112,592 (2435)</td>
<td>83,466 (807)</td>
<td>1.35 (0.04)</td>
<td>108,151 (1773)</td>
<td>80,069 (3951)</td>
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<td>56</td>
<td>100,249 (2763)</td>
<td>73,538 (4241)</td>
<td>1.37 (0.01)</td>
<td>97,554 (354)</td>
<td>68,825 (2034)</td>
<td>1.42 (0.05)</td>
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<td>47,578 (84)</td>
<td>1.58 (0.02)</td>
<td>71,204 (1331)</td>
<td>45,304 (218)</td>
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<td>34,935 (134)</td>
<td>1.56 (0.01)</td>
<td>54,955 (541)</td>
<td>35,378 (322)</td>
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Figure 7. Mechanical properties (stress test) of nonsterilized and γ-irradiated PLCL films (wall thickness: 100 μm) incubated for 0, 30, 60, and 120 days in isotonic PBS, 37 °C, pH 7.4. Testing was conducted at a velocity of 30 cm/min using an Instron Machine. All measurements were normalized relatively to day 0 values obtained for the nonsterilized PLCL films.
compare the \( \gamma \)-irradiation with alternative methods that rely on exposure of the implant to EtO gas. Table V shows the effect of the \( \gamma \)-irradiation method on the initial MW of PLCL balloons compared with EtO-sterilized balloons, and nonsterilized control balloons or bulk polymer. \( \gamma \)-Irradiation of PLCL balloons decreased the polymer’s initial MW by nearly 22%. By contrast, no reduction in initial MW occurred for EtO-sterilized balloons and for nonsterilized ones. Results clearly indicate the advantage of using the EtO method.

In Vivo Feasibility Studies

Dog Study. Initial feasibility testing of the minimally invasive catheter system and the PLCL balloon was conducted on a dog model. The process was simple and intuitive. First, a 3-mm skin incision was performed in the perineum area to separate tissues between the rectum wall and the prostate gland by the injection of 10 mL of saline solution. This preparatory step was followed by the insertion and inflation of the biodegradable balloon. The procedure took less than 8 min, after which the presence of the inflated balloon was ascertained by palpating the rectum wall. Finally, as shown in Figure 10, an X-ray image validated the balloon’s location and its properly inflated form. The dog was followed up for 1 year. During the entire follow-up period, the dog behaved normally, maintained its weight, and appeared viable and in good health. The operated area was completely healed a few days after the procedure, and all monitored physiological parameters were normal, including blood tests. The dog was anesthetized, and during dissection no active inflammatory signs or fibrosis were found. The tissue surrounding the rectum was supple and appeared healthy. The leg of the implanted device (the tube through which the balloon is inflated), 3 mm in size, was found attached to the left anterior side of the rectum, near the apex of the prostate. No signs of the rest of the implanted device were found. Minimal fibrosis tissue was found surrounding the leg of the implanted device, without any sign of active inflammation. The urinary bladder and prostate were macroscopically normal, without any signs of active inflammation or fibrosis. Samples of the prostate were sent to histopathological examination. The pathologist report indicated no systemic toxicity or fibrosis. The balloon was completely biodegraded.

Guinea Pig Study. Four guinea pigs were implanted subcutaneously on with PLCL balloons, as shown in Figure 9.
Following anesthesia, the area of implantation was inflated using 10 mL of saline solution, after which rolled-up balloons were inserted, inflated, and sealed in situ. γ-Irradiation and nonsterilized biodegradable balloons with a wall thickness of 100 μm were used. Although both types were implanted successfully, the durability of the γ-irradiated ones was limited, and all failed 3 days after the procedure. By contrast, nonsterilized balloons remained intact until the animals were sacrificed 2 weeks later. Figure 8(B) shows an implanted γ-irradiated balloon that was removed from a guinea pig 14 days after implantation. These balloons were completely cracked. MW and PDI values of the implants were found to be (72,862 Da, 1.53), similar to the equivalent values obtained in vitro. By contrast, the nonsterilized balloons implanted in the guinea pigs preserved their structure intact for 14 days, at which time the animals were sacrificed and the balloons removed. Figure 8(C) shows one of these balloons, which has preserved its inflated structure and has no cracks on its surface. MW and PDI measurement values of the nonsterilized balloons were found to be consistent with their in vitro analogs (111,620 Da, 1.34). In both cases, the implantation area looked normal, and there was no observable necrosis or edema. Moreover, all animals gained weight and appeared vital throughout the experimental period.

**DISCUSSION**

Patients with prostate cancer who are treated with radiotherapy are going through several radiation sessions over a period that lasts for ~6 weeks. These patients suffer from a variety of permanent adverse effects that degrade their quality of life: damage to normal tissues can result with impotence, bleeding, and uncontrolled urinary function. The novel device described in this study was developed to minimize these outcomes.

The device was developed based on the assumption that by distancing the irradiated prostate from normal, healthy tissues, it is possible to reduce the intensity of peripheral radiation and thereby prevent irreversible damage to these tissues. In addition, by separating the healthy tissues from the cancerous prostate, the intensity of irradiation may be raised without increasing damage to the peripheral environment, which could improve radiation efficiency and shorten the period of treatment.

Distancing organs requires both a distancing element and a methodology of inserting and withdrawing the device. A minimally invasive procedure that uses a biodegradable implant is an optimal solution. The proposed device uses a simple method of catheterization. The implantation procedure is carried out by performing a 3-mm long incision at the perineum. A catheter is then guided by a wire until it reaches the prostate region, proximal to the rectum wall. A rolled up balloon made of a biodegradable polymer is mounted on the middle of the catheter. The balloon is inflated using an isotonic saline solution and sealed in situ with a compatible polymeric plug.

This study focuses on the preparation methodology of the balloon and on its mechanical and chemical evaluation. The mechanical stability of the balloon is the critical requirement that the procedure must meet and the most important element of the present evaluation. Therefore, several criteria were established for the study: First, the balloon polymer must retain its original MW during preparation because of the critical role MW plays in determining the mechanical characteristics of the balloon. As a result, preparation can be conducted only at mild temperatures and cannot be based on popular heating and blowing methods. Second, balloons must be made of an FDA-approved biocompatible material that has a well-known and safe biodegradation pathway. The structural polymer is expected to fully degrade within a period of 1 year, leaving no long-term effects on the surrounding tissues. Third, and most important, balloons cannot have any connecting parts, gluing points or lines, surface defects, or mechanical weak points. Fourth, balloons must be made of highly homogeneous, flexible, and elastic material that retains its mechanical properties during folding. Moreover, the wall thickness of the balloon cannot exceed about 100 μm, as the balloon must be easily rolled up to be inserted through the catheter system. The polymer selected for the preparation of the balloon is poly(lactide-co-e-caprolactone), referred to as PLCL. This biodegradable co-polymer is a well known poly(α,ω-hydroxy acid). PLCL has been used in various medical applications, including drug-loaded films, scaffolds for tissue engineering, and is an FDA-approved material. The PLCL grade (Resomer® LC 703) that was selected has a molar ratio of 70 and 30 for the lactide and caprolactone monomers, respectively. The material is flexible and can be easily rolled up without cracking. Films made of this material were found to have an extremely high-elongation properties of ~700%. (A stress–strain test was conducted using an Instron machine. PLCL films of 1 cm × 4 cm, with a thickness of 100 μm, were withdrawn at a velocity of 30 cm/min).
The mechanical properties of PLCL films reflect the chemical structure of their comprising monomers. The lactide provides toughness, and the caprolactone that has an additional chain of four carbohydrates enhances elasticity. PLCL is an absorbable polymer that degrades hydrolytically at physiological conditions. Its degradation is expected to be complete in about 12 months, and it engages with the body’s natural metabolism, turning into carbon dioxide and water.

The dip-coating balloon preparation technique uses a balloon cast dip coated in a polymeric solution. Agarose gel was selected to serve as the temporary template. Agarose is a simple polysaccharide used as a culture medium and for biological assays. The hydrogel is removed in hot water heated to 90°C that causes the gel to liquefy so it can be pushed through the balloon’s nozzle. Residual agarose is then washed out using hot water aliquots. The agarose template was chosen over organic wax because if dipping were conducted in an organic solution it would either dissolve the template or leave undesired residues on the polymeric film. Although agarose gel is hydrophilic in nature (>95% water w/v), it was found to allow the formation of a polymeric film on its surface when dipped in a PLCL/DCM solution. The polymeric film is continuous and can be thickened by manipulating the process parameters. The dip-coating technique was used to prepare PLCL balloons. Process parameters such as solution concentration, dip speed, and the number of successive coatings were optimized to prepare homogeneous films (Figure 2), with a wall thickness of 100 μm.

Successive coatings increased the balloon’s weight in a nonlinear manner (20 cm/min). Weight contributions were 19, 30, and 51% for the first, second, and third layers. Non-linearity may be partially explained by changes in surface chemical properties during successive coatings. The first layer of the hydrophilic polymer had a limited thickness because it had been applied directly onto a hydrophilic agarose surface. At the same time, the second and third layers were built on an existing polymeric hydrophobic layer that supported their formation.

Nonsterilized PLCL balloons with a wall thickness of 100 μm were found to have the required mechanical stability, as demonstrated by their intactness profiles. Saline-inflated balloons retained their inflated structure, with no weight loss or leaking, after 6 weeks of incubation under physiological conditions (Figure 5). One hundred micrometers was found to be the minimal wall thickness at which balloons do not deflate because of local defects such as local narrowing of the film. At the same time, 100-μm balloons were still sufficiently thin to be easily rolled up to a final diameter of 3 mm, which in turn made them appropriate for use with the standard Seldinger technique.

Nonsterilized PLCL films with wall thickness of 50, 75, and 100 μm were incubated under physiological conditions. After 6 weeks, the films showed moderate weight loss of less than 1%. The moderate decrease is attributed to the initial MW of the polymer (120 KDa) and to its gradual MW degradation profile. More significant weight loss was documented around 140 days, made even more pronounced by film disintegration. Similar PLCL films were tested for their MW loss under physiological conditions. MW values gradually decreased (Table III) regardless of film thickness. This phenomenon is expected because PLCL degrades in a bulk mechanism characteristic of other poly(α-hydroxy acids) as well. Thus, polymer degradation is not strongly correlated with its surface area. Moreover, because PLCL films are so thin, a change in their thickness from 50 to 100 μm probably did not alter significantly the diffusion coefficients for water molecules or degraded polymeric chains that may affect the degradation rate.

Balloons implanted in the body are required to be flexible and withstand occasional mechanical strain. Therefore, loss of their mechanical properties is directly related to their proper functioning. The mechanical properties of the PLCL films (wall thickness: 100 μm) were characterized using an Instron machine in stress–strain mode. All measured parameters demonstrated the gradual decrease in the mechanical properties of the polymer over time (Figure 7). The change in the mechanical properties is associated with the decrease in the polymer’s MW. Despite the gradual decrease in mechanical properties, films were left with sufficient mechanical strength after 6 weeks of incubation, with an ultimate stress of ~63% relative to the original value, and ~400% of elongation, a very high value. A preliminary in vivo study tested nonsterilized PLCL balloons implanted subcutaneously in guinea pigs. Balloons removed 14 days after implantation (Figure 9) preserved their inflated form (Figure 8).

Every implantable medical device is required to undergo a sterilization step. This step is critical for biodegradable polymers because if they are not properly sterilized, entrapped organisms may be released during degradation. Various sterilization methods are routinely used for medical devices. Among the most popular are autoclaving, EtO exposure, and γ-irradiation. The sterilization procedure must take into account the characteristics of the device, including its chemical, physical, and mechanical properties. γ-irradiated PLCL balloons were tested for their intactness profiles using inflated balloons filled with isotonic saline, either incubated under physiological conditions or implanted subcutaneously in guinea pigs. Samples were monitored weekly for weight and possible leakage. Both in vitro and in vivo results indicated that γ-irradiation is inappropriate. Inflated PLCL balloons failed in less than 3 weeks of incubation in physiological buffer (Figure 5). Similarly, the in vivo samples implanted in guinea pigs deflated after 1 week (Figures 9 and 10). These results contrasted with the nonsterilized balloons that survived more than 6 weeks in their inflated form.

To gain deeper insight into this phenomenon, PLCL balloon films were studied extensively for their mechanical (Figure 7), weight loss (Figure 6), and MW loss profiles (Table VI). The in vitro tests were carried out after incubation under physiological conditions. The decline in the flex-
ibility (% of elongation) of the γ-irradiated PLCL balloons demonstrates the loss of their mechanical properties. Films lost their entire flexibility after 30 days of incubation in physiological buffer, compared with the gradual decrease of 25% that was observed in nonsterilized samples. This change can probably be attributed to the substantial decrease in the films’ initial MW (~35%). In addition, γ-irradiated films showed enhanced degradation rate, with a nearly 1.5-fold increase in MW loss profile and an ~twofold increase in weight loss profiles over nonsterilized films. Moreover, γ-irradiated films disintegrated twice as fast as did nonsterilized samples and showed longitudinal cracks (Figure 8). The extreme decline in the chemical-physical properties of γ-irradiated PLCL films explains their short intactness profile. Autoclave, an alternative sterilization technique, is not applicable to biodegradable polymers because it is carried out in harsh conditions, including extreme temperatures, elevated pressure, and humidity. Steaming can cause rapid degradation, and the high temperatures can alter the form of the films as the polymer is heated above its glass transition temperature ($T_g$). Among the sterilization technologies currently available to the medical device industry, EtO is highly popular. The EtO process is based on exposure of the implant to EtO, which eliminates all living organisms. Typically, the EtO process can be broken down into several steps (air removal, steam injection, gas injection, and purge), each needs careful planning to ensure a safe and effective process. This methodology is usually less preferable than γ-irradiation because it requires a much longer validation procedure. In this study, the EtO method was evaluated after concluding that γ-irradiation is inappropriate for PLCL balloons, and a sterilization method was sought that does not deteriorate the polymer’s MW, which in turn determines its mechanical properties and the intactness profile of the balloons. PLCL balloons and bulk PLCL polymer were sterilized using the EtO method and evaluated for their MW (Table V). Sterilized samples were found to retain their MW similarly to controls. Additional tests using EtO-sterilized balloons were recently conducted in our laboratory (data not shown) and PLCL balloons were implanted using a swine model. The objective of the study was to evaluate the clinical aspects of radiation therapy on the surrounding tissues.31 EtO-exposed balloons were removed from the animals after 60 and 90 days. In contrast to the γ-irradiation destructive effects, the external beam radiation (X-ray) did not damage balloon’s mechanical stability. Accordingly, the EtO sterilization method was selected for the PLCL balloons.

PLCL balloons were successfully implanted in both dog and guinea pig models. The minimally invasive catheter system was functional and enabled easy and reliable insertion and sealing of the balloons. In the dog model, the procedure was similar to the one designed for humans (Figure 1). The entire process was found to be simple, without complications, and it lasted less than 10 min. Degradation time of balloon films with 100-μm wall thickness was less than a year and left no traces of PLCL film at the implantation site. However, the balloon plug and its surrounding sleeve, also made from a PLCL polymer, were located within the implanted tissues. Because the plug and its surrounding sleeve generate a cylinder with a diameter of 3 mm, it was expected to have an extended degradation time compared with the balloon film. The substantial decrease in diffusion coefficients for water and for degradation products may be responsible to the extended degradation time of the polymer. PLCL has been previously approved by the FDA for human use,32 and it is therefore not surprising that no

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Table VI. Molecular Weights Loss for γ-Irradiated 100-μm Balloons

Figure 10. X-ray image taken 1 h post implantation of a PLCL balloon in a dog model. A balloon that was rolled up on its catheter needle was inserted throughout the perineum, inflated with saline, and then sealed in situ (Figure 1).
adverse affect were found locally in the dog a year after implantation. The proposed method can be further explored both in vivo and in vitro to demonstrate safety and effectiveness in human trials.

Implanting a biodegradable balloon that retains its mechanical stability for a prolonged period of time may be used in other applications as well. Catheterized balloons may serve as a reservoir for drug delivery, with specific drugs and dosages easily tailored through the inflation medium. In surgical interventions, it can be used to distance organs during the wound healing process, especially in orthopedic applications.

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REFERENCES